



Studies on combined chemo-mechanical pretreatment on fish waste for biofuel production

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Abstract

In this experimental study chemo-mechanical pretreatment is carried out for 3 hours and samples are collected at equal interval of time. The pretreatment efficiency is evaluated based on COD solubilization and SCOD release, protein and carbohydrate. The fish waste sample was collected and homogenized using convectional mixer for uniformity of the sample. Then the effective disperser 14000rpm was optimized in 60 minutes and the optimized chemo disperser rpm will be combined with 1N of NaSO₄ to achieve desired sample with dosage variation between (25 – 100) µL and 30 minutes were found. The higher COD solubilization 18% and SCOD release at 2950 mg/L was achieved. Then the bio fuel assay will be conducted to evaluate the pretreatment performance. Finally, the biomethane production was found with the release of SCOD, COD, protein and carbohydrate were found to be maximum. The combined chemo disperser pretreatment of fish waste were found.

Keywords: pretreatment, protein, carbohydrate, fish waste

1. Introduction

The world marine capture fisheries contribute more than 50% of the total world fish production. About 70% of fish is processed before final sale, resulting in 20-80 % of fish waste depending on the level of processing and type of fish. In addition, a significant amount of the total catch from fish farming is discarded each year. Also, fish processing operation require large volumes of potable water which results in significant amounts of waste water. The majority of fish wastes are disposed in the ocean. The aerobic bacteria present in the water breakdown the organic matter in the presence of oxygen leading to a considerable reduction of oxygen in water.

Achievement of fishery production is supported by the contribution of aquaculture production which it continues to rise, reaching 11.13% per year. The raising of fishery production is accompanied by increased range of value-added processed products that produced by the Fish Processing Unit (UPI), both large and small (household) scale to meet consumer preferences towards diversification of fishery products in good quality and safe for consumption and have added value.

A new energy source as abundant renewable energy alternative to fossil fuels. One of these is the use of alternative energy biogas. Biogas can be categorized as bio-energy, because the energy produced from biomass. Biomass is organic material is relatively young ages derived from living or products and industrial wastes cultivation (agriculture, farming, forestry, animal husbandry and fishery). Biogas is gas end products digestive/anaerobic degradation (in environments without oxygen) by bacteria methanogen. Without a good fishery waste management, it will result in the destruction of the environment polluted by organic waste. Organic waste that mixed with water will result in decreased water quality, environmental and social impacts.

2. Literature reviews

Mshandete *et al.*, 2004 ^[9] have been studied about anaerobic batch co- digestion of sisal pulp and fish wastes, Co-digestion of various wastes has been shown to improve the digestibility of the materials and biogas yield. Batch wise digestion of sisal pulp and fish waste was studied both with the wastes separately and with mixtures in various proportions. While the highest methane yields from sisal pulp and fish waste alone were 0.32 and 0.39 m³ CH₄/kg volatile solids (VS), respectively, at total solid (TS) of 5%, co-digestion with 33% of fish waste and 67% of sisal pulp representing 16.6% of TS gave a methane yield of 0.62 m³ CH₄/ kg VS added. This is an increase of 59–94% in the methane yield as compared to that obtained from the digestion of pure fractions at 5% TS. Nges *et al.*, 2011 ^[10] have been studied about improved utilization of fish waste by anaerobic digestion following omega-3 fatty acids extraction, Fish waste is a potentially valuable resource from which high-value products can be obtained. Anaerobic digestion of the original fish waste and the fish sludge remaining after enzymatic pre-treatment to extract fish oil and fish protein hydrolysate was evaluated regarding the potential for methane production. The results showed high biodegradability of both fish sludge and fish waste, giving specific methane yields of 742 and 828 m³ CH₄/tons VS added, respectively. However, chemical analysis showed high concentrations of light metals which, together with high fat and protein contents, could be inhibitory to methanogenic bacteria. The feasibility of co-digesting the fish sludge with a carbohydrate rich residue from crop production was thus investigated, and a full-scale process outlined for converting odorous fish waste to useful products.

Regueiro *et al.*, 2012 ^[11] have been studied about enhanced methane production from pig manure anaerobic digestion using fish and biodiesel wastes (BW) as co-substrates, Co-

Digestion of pig manure (PM) with fish and biodiesel waste was evaluated and compared with sole PM digestion. Results indicated that co-digestion of PM with FW and/or BW is possible as long as ammonium and volatile fatty acids remained under inhibitory levels by adjusting the operating conditions, such as feed composition, organic loading rate (OLR) and hydraulic retention time (HRT). PM and FW co-digestion (90: 10 and 95: 5, w/w) was possible at OLR of 1–1.5 g COD/L d, resulting in biogas production rates of 0.4–0.6 L/L d and COD removal efficiencies of 65–70%. Regarding BW, good results (biogas production of 0.9 L/L d and COD elimination of 85%) were achieved with less than 5% feeding rate. Overall, operating at the same OLR, the biogas production and methane content in the co-digester was higher than in the only PM digester. Ferguson *et al.*, 2016 have been studied about the anaerobic digestion of fish wastes. A method and apparatus for anaerobic digestion of fish wastes and production of useful by-products including fertilizer and biogas. The method includes grinding up the fish wastes and adding liquid to create a suspension of up to 25% solids, optionally adding lipase to break down the fish oil and an aerobically digesting the fish suspension over a period of approximately 20 days at a temperature in the range of 35°-55°C. With mixing provided by recirculation or other means. After digestion, the suspension is pumped to a secondary chamber for storage, further breakdown by mechanical means such as agitation or recirculation, centrifugation to remove suspended solids, production of an enhanced fertilizer by the addition of seaweed, urea, nitrogen, potash and chelated micronutrients, deodorization, by the addition of potassium permanganate, treatment to reduce the B.O.D. or evaporation to reduce liquid.

3. Materials and Methods

The lab study was carried out to analyze the effect of chemo-mechanical pretreatment of fish waste for biofuel production. The optimum conditions for chemo mechanical pretreatment have to be investigated prior to biofuel potential.

Chemicals Used

The chemicals used in this research work were listed as below.

- Folins reagent
- Anthrone reagent
- Sodium hydroxide
- Ferrous ammonium sulphate
- Sulphuric acid
- Lowry's reagent
- Potassium dichromate
- Mercuric sulphate

3.1 Methodology

The schematic representation of methodology of the work shown in figure

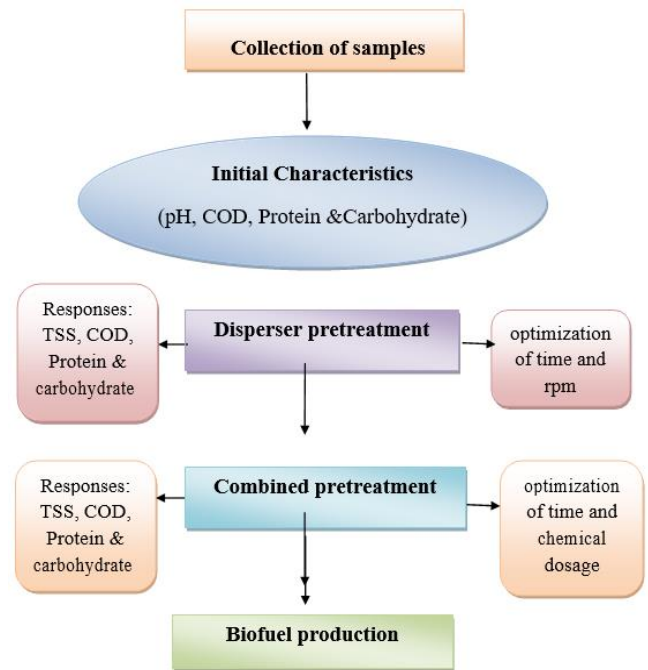


Fig 1: Schematic Representation of Methodology

3.2 Collection of Sample

Fish waste collected on a fish market from Tirunelveli Town. Collection of waste was shown in figure 3.2. It includes leftover fish consisting of heads, tails, skin, gut, and frames. The fish waste was mixed with the kitchen blender. It was shredded into a size of 1-2mm and stored at 4°C in a refrigerator.



Fig 2: Collection of Fish Waste

3.3 Initial Characteristics of Fish Waste

The initial characteristics of FW includes suspended solids, total chemical oxygen demand (TCOD), Protein, Carbohydrate, pH was analyzed by using standard reference as mentioned in table 3.1 (APHA, 2005). The performance has been detailed in the following sections.

Table 1: Standard Reference for Initial Characteristics

S. No	Parameters	References
1	Suspended solids	APHA, 2540 D
2	TCOD	APHA, 5220 D
3	SCOD	APHA (2005)
4	pH	4500-H, APHA (2005)
5	Protein	Kavitha <i>et al</i> , 2019
6	Carbohydrate	Kavitha <i>et al</i> , 2019

3.4 Disperser Pretreatment

A series of experiments will be performed with disperser (IKA T25 Ultra Turrax Digital disperser) at different working rpm range (4000-24000) in order to enhance solubilization of particulate material. Disperser which is shown in figure 3.3 is a high-speed shearing device to disperse large solids into small particles. Due to the high rotation speed of the rotor, the medium to be processed in automatically drawn axially into the dispersion head and then forced radially through the slots in the rotor/stator arrangement. In addition, high turbulence occurs in the shear gap between rotor and stator, which provides optimum mixing of the suspension. For each experiment, fish waste and distilled water in the ratio of 1:10 will be placed in a glass beaker without temperature adjustment and disperser probe to be submerged in the sample to a depth of 2cm above the bottom of the beaker. Samples will be collected at different time intervals.



Fig 3: Photographic View of Disperser

3.5 Chemo Mechanical Pretreatment

The chemo mechanical pretreatment was carried out by the pH variation and optimized disperser working conditions which is shown in table 3.2. During the course of experiment samples will be collected and analyzed at regular time intervals.

Table 2: Condition to be Tested

S. No	Parameter	Range
1	pH	8-12
2	rpm	4000-24000
3	time	0-180mins

3.6 Biomethanation Assay

Biofuel potential is performed to evaluate potential for

biogas recovery from fish waste after pretreatment of disperser. The biodegradability assays will be conducted in anaerobic batch reactors of 300 mL capacity serum bottles A, B, C respectively. Biofuel potential setup was shown in figure 3.4. Each serum bottle will be filled with 150 mL of inoculum and 50 mL of substrate. A control treatment with 150 mL of inoculum will be mixed with 50 mL of untreated fish waste and it will be used to determine biofuel production due to endogenous respiration. The inoculums needed to be quite active, with a good adaptability and a low endogenous respiration. For these experiments, rumen bacteria from the digestive tract of a cow were used as an inoculum. Rumen fluid contains high amounts of anaerobic bacteria. A higher quantity and more species of anaerobic bacteria in rumen fluid enable the degradation of more kinds of substrates. The rumen fluid seeded in a bio digester has a significant effect on the cumulative biofuel production rate. The initial pH was recorded as neutral before the start of biofuel assay tests. After adding the substrates and inoculum, the reactors were closed with a rubber septum and an aluminum seal to make them air tight and was subsequently purged with nitrogen gas at the rate of 10 mL per second for 20 min into the reactors to maintain anaerobic conditions. An internal temperature of 35°C was maintained by incubating the reactors in a temperature controlled mechanical shaker (220 rpm). The biofuel was measured by inserting a needle into the septum. Gas accumulation produced during the incubation was controlled using a syringe. The syringe piston was pushed up due to the increase of pressure inside the bottle when the gas was produced, and the displaced volume was recorded.

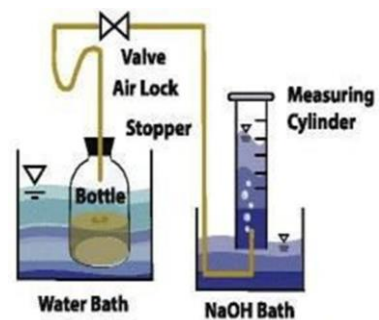


Fig 4

4. Results and Discussion

This experimental test results about the experimental outcome of lab scale study conducted based on proposed methodology. It includes, initial characterization of Fish waste, optimization value, treatment time and chemical dosage, evaluating the effect of combinative chemo-mechanical pretreatment of fish waste. The experimental data of lab scale study was explained in the following section. The initial characteristics of fish waste was shown in a table 4.1

Table 3: Initial Characteristics of Fish Waste

Sl.no	Parameters	Value	Units
1	pH	7.5	-
2	Total Chemical Oxygen Demand	88000±380	mg/L
3	Soluble Chemical Oxygen Demand	400±120	mg/L
4	Suspended Solids	50000±150	mg/L
5	Protein (OD)	0.135	-
6	Carbohydrate (OD)	0.057	-

The process was known for its effectiveness in terms of SCOD release. The disperser pre-treatment was predicted by measuring the SCOD release during the disperser pre-treatment process. rpm is the major governing factor for disperser pre-treatment. In the Figure, illustrates the influence of rpm (1000- 16000) and treatment time (0-120min) on SCOD releases. The release of SCOD during disperser pre-treatment was caused by rotor-stator effect. The effect of this disperser pretreatment on SCOD release was investigated and depicted in table 4.2 and figure 4.1. It was observed that the SCOD release increased progressively with increase in temperature, pH, and time indicating the solubilization of most of the particulate organic matters into soluble organics.

Table 4: Effect of SCOD release at different disperser pretreatment

SCOD release (mg/L)				
Time	1000rpm	12000rpm	14000rpm	16000rpm
0	300	300	300	300
10	344	378	416	458
20	594	653	719	791
30	713	784	862	948
40	863	949	1044	1148
50	1125	1238	1361	1498
60	1219	1341	1475	1623
90	1250	1375	1513	1664
120	1406	1547	1702	1872

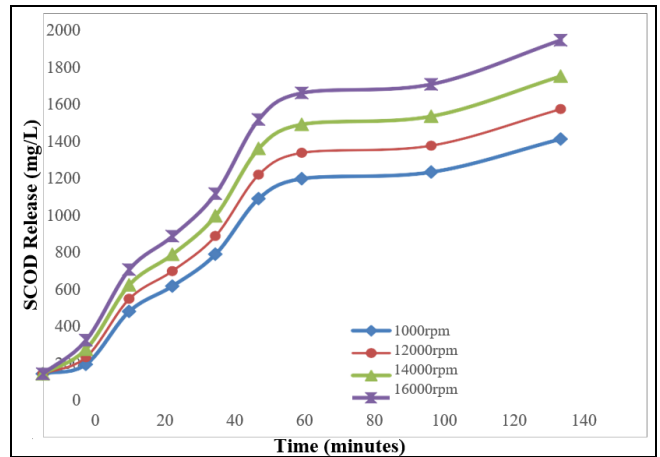


Fig 5: Effect of SCOD at different disperser pretreatment

The effect of COD solubilization was illustrated in figure 4.2 and table 4.3. Solubilization defines as the ratio of soluble organics to total organics. The released soluble organics will be utilized by the methanogenic anaerobes as substrates to increase the methane production. The effect of COD solubilization was analyzed for the various time interval between (0 – 120) min and various disperser rpm range between (1000 – 16000) rpm. Irrespective of time, rpm plays a major role in enhancing fish waste solubilization. The effect of COD release at the optimum time is 60 min and the disperser is 14000rpm respectively.

Table 5: Effect of COD solubilization at disperser pretreatment

COD Solubilization (%)				
TIME	1000rpm	12000rpm	14000rpm	16000rpm
0	1.875	1.875	1.875	1.875
10	2.15	2.3625	2.6	2.8625
20	3.7125	4.08125	4.49375	4.94375
30	4.45625	4.9	5.3875	5.925
40	5.39375	5.93125	6.525	7.175
50	7.03125	7.7375	8.50627	9.3625
60	7.61875	8.38125	9.21875	10.1438
90	7.8125	8.59375	9.45625	10.4
120	8.7875	9.66875	10.6375	11.7

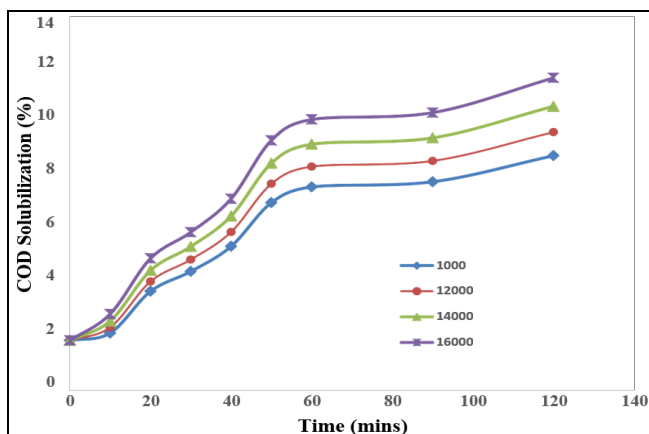


Fig 6: Effect of COD solubilization at disperser pretreatment

4.1 Protein Release during Disperser Pretreatment

Protein was considering as additional evaluating parameter, to evaluate the effect of disperser pretreatment on fish waste. Protein was evaluated for different rpm as 1000, 12000, 14000, 16000 and different time 0, 10, 20, 30, 40, 50, 60, 90, 120 min respectively. The protein release was increased in 60 min. Further increase in the rpm causes decreasing effect in protein. As expected, the concentrations of these soluble organics were much higher in fish waste at optimum condition. The maximal values of protein released were 0.549, 0.549, 0.664, 0.730 for rpm 1000, 12000, 14000, 16000 respectively. The results were in accordance with the results of SCOD release and COD solubilization. Table and figure 4.3 show the effect of protein release at different rpm at optimum time 60 min.

Table 6: Effect of protein release at disperser pretreatment Vs time

Time	Absorbance (OD)			
	1000rpm	12000rpm	14000rpm	16000rpm
0	0.135	0.135	0.135	0.135
10	0.155	0.17	0.187	0.206
20	0.267	0.294	0.324	0.356
30	0.321	0.321	0.388	0.427
40	0.388	0.388	0.47	0.517
50	0.506	0.506	0.612	0.674
60	0.549	0.549	0.664	0.73
90	0.563	0.563	0.681	0.749
120	0.633	0.696	0.766	0.842

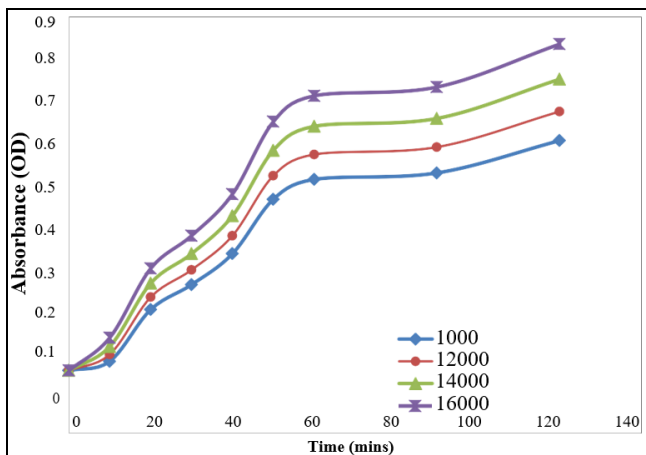


Fig 7: Effect of protein release at disperser pretreatment Vs time

4.2 Effect of Carbohydrate Release on Disperser Pretreatment

Carbohydrate was considering as additional evaluating parameter,

to evaluate the effect of disperser pretreatment on fish waste. Carbohydrate is evaluated for different rpm such as 1000, 12000, 14000, 16000 respectively. The samples were collected at different time interval 0, 20, 40, 60, 80, 100, 120 min. The maximal values of carbohydrate released were 0.232, 0.255, 0.280, 0.308. Table 4.5 and figure 4.4 shows the effect of carbohydrate release at different rpm.

Table 7: Effect of carbohydrate release at disperser pretreatment

TIME	Absorbance (OD)			
	1000rpm	12000rpm	14000rpm	16000rpm
0	0.057	0.057	0.057	0.057
10	0.065	0.072	0.079	0.087
20	0.113	0.124	0.137	0.15
30	0.135	0.149	0.164	0.18
40	0.164	0.18	0.198	0.218
50	0.214	0.235	0.259	0.285
60	0.232	0.255	0.28	0.308
90	0.238	0.261	0.287	0.316
120	0.267	0.294	0.323	0.356

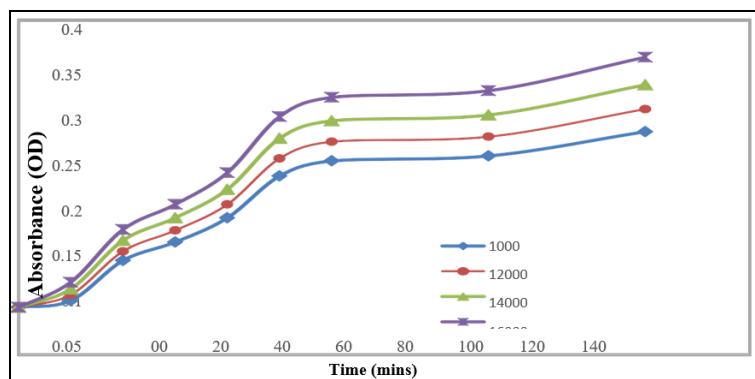


Fig 8: Effect of carbohydrate at disperser pretreatment Vs time

4.3 Effect of Ph Release at Disperser Pretreatment

The effect of pH can be illustrated in figure 4.5 and table 4.6. It explains the effect of disperser pretreatment at

various intensities for different time by pH release. The pretreatment observed that the disperser intensity increases to decrease pH. The maximum pH obtained in 60 min.

Table 8: Effect of pH release at disperser pretreatment

Sl. No	Time	pH
1	0	7.46
2	10	7.47
3	20	7.47
4	30	7.5
5	40	7.52
6	50	7.54
7	60	7.57
8	90	7.59
9	120	7.6

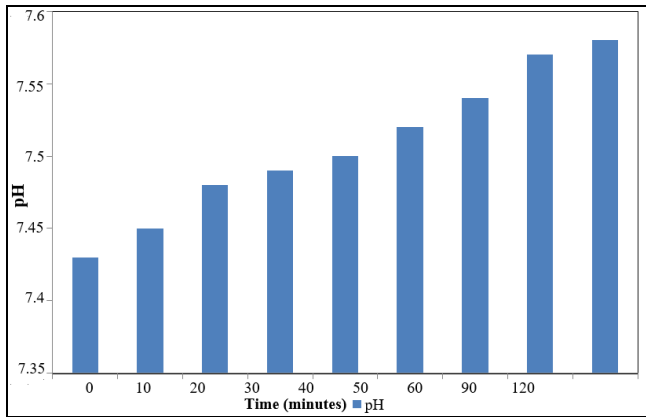


Fig 9: Effect of pH at different disperser pretreatment

4.3 Temperature Release on Disperser Pretreatment

The effect of temperature was investigated at different disperser rpm and time min. Table 4.7 and figure 4.6 explains the effect of disperser pretreatment with different time interval (0-120) min and different rpm (1000-16000) respectively.

Table 9: Effect of temperature at disperser pretreatment

Sl. No	Time	Temperature (° C)
1	0	31
2	10	32
3	20	33
4	30	34
5	40	35
6	50	36
7	60	37
8	90	38
9	120	39

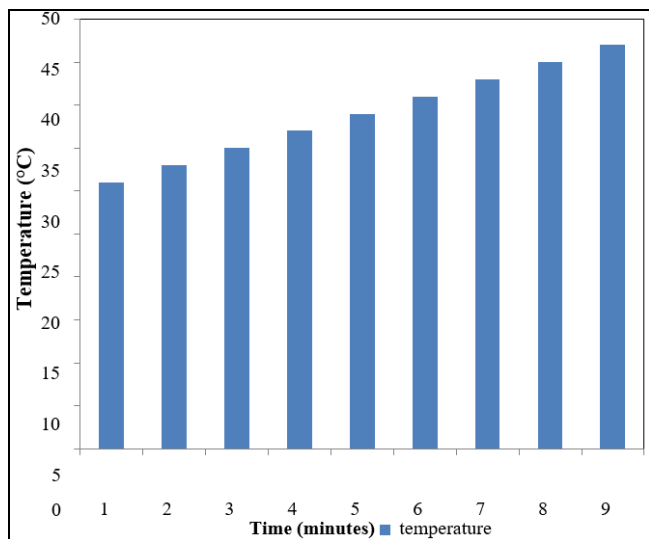


Fig 10: Effect of temperature at different disperser pretreatment

Conclusions

- Waste generated from fish processing plants varies from 10 to 50% of landed fish depending on the type of fish, product and processing techniques.
- Fish plants in India produce approximately 18000 tonnes of waste per year, most of which is discharged to the ocean and sent to landfill after treatment.

- The degree of processing depends on the region, type of harvesting, and type of fish. High biodegradability and organic matter make fish waste suitable for anaerobic digestion (AD) for effective biofuel production.
- Biofuel helps to reducing the emission of gases which leads to global warming. The hydrolysis is the limiting step for anaerobic digestion in order to enhance rate of hydrolysis pretreatment is applied.
- It was planned to pretreatment the fish waste through combinative chemo disperser pretreatment. It has been selected as an combined effect to bring about synergistic effect in waste reduction and biofuel production.
- In this phase, the disintegration of FW by disperser pretreatment and the chemo disperser pretreatment and the biodegradability studies was carried out.
- The optimal value of disperser pretreatment is 14000rpm, time 60 mins, the SCOD release at 1475 mg/L and the COD solubilization was 9.2%. The chemo disperser was carried out using optimized disperser rpm (14000) and varying chemical dosage from (25 – 100 µL). At optimal dosage (75µL), the COD solubilization was 18% and SCOD released of 2950 mg/L were achieved.
- In this combined chemo disperser pretreatment of SCOD release, protein and carbohydrate were found to be maximum. A highest methane production was observed for disperser and chemo disperser pretreatment was 192 and 386 mL of methane respectively.

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